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Effect of pH changes on the growth of water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) in acid mine drainage

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Abstract

Acid mine drainage (AMD) is waste water formed through a series of chemical reactions and biological activity. AMD has a pH of 3.0 and limited nutrients content. Therefore, before being released into the environment, it requires pH modification to deserve for growing of aquatic plants. This study aimed to determine the effect of pH on the growth of water hyacinth. AMD was collected from coal mining in Tanah Laut, South Kalimantan and limestone was taken from District of Tarjun, Pulau Laut. The 10 L of AMD add with limestone: 50 g, 100 g, and 150 g left for 3 days and pH turned into 4.01 (A_1); 4.36 (A_2); and 4.87 (A_3), with 3 replications. Measured parameters were AMD pH changes and growth of water hyacinth (total weight, root length, and diameter of leaf), every 3 days after growth. The results showed that pH changes significantly affect the growth of total weight (R = 0.909), root length (R = 0.901), and diameter of leaf (R = 0.918) of the water hyacinth. The highest growth in total weight and root length occurred in the day 9, pH increased from 5.64 to 5.91, and the diameter of leaves on the plant occurred in the day 12 and a pH of 5.28 to 5.48. The reduced rate of growth of water hyacinth at a pH closer to neutral state can be due to the reduced availability of nutrients, as AMD containing low nutrients.

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Introduction

Active and abandoned coal mining with open system (overburden) are a major pollution concern in South Kalimantan. In addition, this system may alter terrestrial ecosystems and produce wastes (tailings and rock) contain sulfide minerals, such as pyrite (FeS_2) called Acid Mine Drainage or (AMD). This AMD occurs as a result of pyrite oxidation reaction which will form ferrous ions (Fe 2+), sulfate (SO42-), and some of the protons forming acidity that lead the environment becomes acidic conditions (Watzlaf *et al.*, 2004). The reaction is accelerated by microbes known as extremophiles. This microbe such as *Acidithiobacillus ferrooxidans* is capable living in adverse conditions (low pH) of natural rocks with limited water and oxygen. Thus the reaction causes changes in acidity to pH <5. These conditions may increase the solubility of heavy metals into the water. This process is maximal at pH 2-4. One source of AMD in South Kalimantan has a pH of 3.0 and containing heavy metals. Several heavy metals have been found in the AMD as Fe, Mn, Pb, Cd, Hg, As, Al, Cr, Ni, Zn, Co, Cu (Gomes *et al.*, 2006; Blodau, 2006). Agarwal (2009) and Merrill *et al.* (2007) reported that Cd, Hg, As, Cr, Zn, and Pb are toxic metals that harmful to organism and environmental. Thus the acid water disposal can cause water pollution. Therefore, it is important to seek the effort to overcome the above problem through phytoremediation e.g. using water plant.

Water hyacinth (*Eichhornia crassipes*) is a noxious weed that has attracted worldwide attention due to its fast spread and rapid growth, which sometimes lead to serious problems in navigation, irrigation, and power generation. Because it's characteristic this plant are used as phytoremediation agent. This plant is able to grow on nutrient with pH, temperature, and toxic materials. It is also known to have the potential to clean up wastewater or hiperaccumulator known as heavy metal. Lu (2009) reported that most plants grow best in a pH range of 5.5 to 7.0. Besides, El-Gendy *et al.* (2004), reported that the optimum pH for growth of water hyacinth is neutral the plant is tolerate at pH 4-10. Effect of pH on the plant is more detailed reported by Trinidad *et al.* (2008), that the maximum growth (number of plants and dry weight) is at pH 7, while at pH of 3.2-4.2 was highly toxic to plants, stunted growth 4.2-4.3, and 4.3-4.5 may be hampered.

Preliminary studies have shown that water hyacinth can not adapt and cause chlorosis on leaves, stems, and roots loss when expose in acid mine water with a pH of 3.0. This is in line with Ratnaningsih *et al.* (2010) study, states that the majority of aquatic plants die because they can not tolerate low pH (pH <4).

The objective of this study is to determine the effect of pH on the growth of water hyacinth exposed in acid mine drainage. The benefits of this research is to provide scientific information regarding the growth of water hyacinth plants in AMD that may later be applied as an agent or accumulators of heavy metal phytoremediation.

Material and methods

Study Site

This research was conducted at the Laboratory of Elementary Mathematics and Natural Sciences University Mangkurat Banjarbaru in April-September 2013. Acid mine water was collected from one of the locations of coal mining in Tanah Laut, South Kalimantan.

Data Collection

Limestone was taken from the District Tarjun Sea Island District. Limestone was made uniform with a diameter of 2 cm. Water hyacinths were isolated from swamp land in Banjar, South Kalimantan. The collected water hyacinths were selected with criteria as follows: have 15-20 cm height, leaf number between 4-6 sheets, and a total individual weight of 60-80 g (after cleaning). The plants acclimatized in the aquarium provided with nitrogen fertilizer phosphorus and potassium until reproduce new tillers prior to experiment.

Experiment

The 10 L of acid mine water (pH 3.0) put in pots and add with 3 different amounts of limestone (calcium carbonate) as follows: 50 g, 100 g, and 150 g. The solution was left for 3 days and the pH turned into 4.01 (A1); 4.36 (A2); and 4.87 (A3). Tillers of water hyacinth in the aquarium were cleaned and dried. The root length, leaf diameter and total weight were measured. Hyacinth planted in pots containing 3 acid mine water variations. These treatments were done with 3 replications. Parameters measured were AMD pH changes and growth of water hyacinth (total weight, root length, and the diameter of the leaf. Measurements were performed every 3 days after growth.

Data analysis

Absolute Growth Rate

The growth of root length and diameter of the leaf were converted into absolute value of the growth rate or the Absolute Growth Rate (AGR). Absolute Growth Rate is index to calculate magnitude of growth rate every 3 days. This index is calculated by the formula as follows (Demchik and Garbutt, 1999):

$$AGR = \frac{X_2 - X_1}{t_2 - t_1}$$

Description:

AGR = Absolute Growth Rate

X_1: root length / diameter leaf on t_1

X_2: The length of the root / leaf diameter on t_2

t_1: Time early planting

t_2: Time after 3 days of growth

Relative Growth

Data growth was converted into the value of the total weight of the relative growth or Relative Growth (RG) with the formula Mohamad and Puziah (2010):

$$RG = \frac{\textit{Final fresh weight (FFW)}}{\textit{Initial fresh weight (IFW)}}$$

Description:

RG = Relative Growth

FFW: total weight of water hyacinth on each additional age 3 days

IFW: total weight of the water hyacinth in the early planting

Statistical Analysis

Effect of pH on the growth of the total weight, root length, and the diameter of the leaves were analyzed by linear regression method correlation raw data (before averaged) with SPSS (Statistical Product and Service Solutions) version 20.

Result and discussion

The average result of measurements of pH and growth of water hyacinth (total weight, root length, and the diameter of the leaves) as well as the calculation of the third AGR, RG and acid mine water pH variation during the 21 days of treatment are presented in Table 1.

Table 1. The change in pH, water hyacinth growth, and RG calculation and AGR on AMD during the 21 day treatment.

Treatment	Parameter	Age (Days)							
		0	3	6	9	12	15	18	21
A1	pH	4.01	4.29	4.48	4.67	4.90	5.08	5.22	5.32
	BT (g)	14.92	17.62	21.21	30.90	43.51	60.72	75.90	84.60
	RG BT		1.18	1.20	**1.46**	1.41	1.40	1.25	1.11
	PA (cm)	1.18	2.26	4.87	8.77	12.40	15.83	18.97	20.13
	AGR PA		0.36	0.87	**1.30**	1.21	1.14	1.05	0.39
	DD (cm)	2.38	3.03	3.93	5.83	8.83	11.57	14.53	15.20
	AGR DD		0.22	0.30	0.63	**1.00**	0.91	0.99	0.23
A2	pH	4.36	4.85	5.07	5.28	5.48	5.67	5.83	5.95
	BT (g)	15.62	20.58	34.22	57.30	80.56	95.90	107.98	116.50
	RG BT		1.32	1.66	**1.67**	1.41	1.19	1.13	1.08
	PA (cm)	1.28	3.07	6.13	12.67	15.50	20.83	23.23	25.47
	AGR PA		0.60	1.02	**2.18**	0.94	1.78	0.80	0.75
	DD (cm)	2.37	3.05	4.53	7.10	12.57	15.93	18.33	20.52

Treatment	Parameter	Age (Days)							
		0	3	6	9	12	15	18	21
A3	AGR DD		0.23	0.49	0.86	**1.82**	1.12	0.80	0.73
	pH	4.87	5.33	5.64	5.91	6.22	6.49	6.70	6.85
	TW (g)	15.50	24.83	41.80	72.62	98.50	122.80	139.66	148.46
	RG of TW		1.60	1.68	**1.74**	1.36	1.25	1.14	1.06
	RL (cm)	1.27	5.20	10.17	16.97	22.52	27.44	30.92	33.92
	AGR of RL		1.31	1.66	**2.27**	1.85	1.64	1.16	1.00
	LD (cm)	2.37	4.87	9.83	14.24	18.50	23.23	25.73	26.90
	AGR of LD		0.83	**1.65**	1.47	1.42	1.58	0.83	0.39

Note: A1 = acid mine water add with 50 g of limestone, A2 = acid mine water add with 100 g of limestone; A3 = acid mine water add with 150 g of limestone; TW = total growth; RL = root length; LD = leaf diameter; RG = Relative Growth*;* ARG = Absolute Growth Rate.

Table 1 showed that of the three treatment of acid mine water for 21 days, the pH increased respectively by 32.67% on the A_1 (4.01 to 5.32), A_2 amounted to 36.47% (4.36-5.95) and A3 by 40.66% (4.87-6.85). This change suggests that the difference in the quantity of limestone lead to increase pH. Such improvement was caused by different limestone surface area, so that the reaction chamber between the acid carrier compound and neutralizing compound has increased. Younger *et al.* (2002) reported that limestone and calcite are able to increase the pH of the water by using hydrogen ions through ion adding bicarbonate alkalinity, with the following reaction:

$CaCO_3 + H^+ \rightleftharpoons Ca^{2+} + HCO_3^-$ (1)

$HCO_3^- + H^+ \longrightarrow H_2O + CO_2\,(aq)$ (2)

Thus more calcium carbonate was ionized; the higher will pH increase or neutralization process faster. Another factor is the presence of water hyacinth. This plant is able to improve the waste water pH (acidic conditions) or lower the pH (alkaline conditions). Water hyacinth can increase the pH from 4.2 to 7.4 for 8 days in effluent water out Ratnaningsih *et al.* (2010). In addition to the above factors, the presence of microorganisms can also cause an increase in the pH of the wastewater. Ulfa *et al.* (2013), has identified the presence of sulfate reducing bacteria *Thiobacillus* sp and *Pseudomonas* sp on AMD. Drury (2006), states that sulfate reduction is the main factor in the pH neutralization, sulfate and toxic metals reduction, in waste water. Sulfate reduction is a biochemical process in which sulfate reduced to sulfide and bicarbonate. This process produces bicarbonate alkalinity (Greben *et al.*, 2005). The process of increasing the pH by sulfate reducing bacteria, which begins with the presence of organic matter (CH_2O) as a carbon source for growth and sulfate ions as electron acceptors. The reaction of the process of bicarbonate production is as follow:

$SO_4^{2-} + 2CH_2O \rightleftharpoons H_2S + 2\,HCO_3^-$ (3)

Base on the above reaction, production of bicarbonate lead to the faster the acid neutralization. It is clear that the pH changes were faster in early treatment, but slower in the latter.

Total Weight

The total weight is determined by weighing of the entire plant in a fresh condition (immediately after harvest). Table 1 showed that of the three different treatments for 21 days, the growths of the total weight of the water hyacinth were increased in all treatment (Fig. 1). The total weight in treatment A_1 has increased 69.68 g (14.92 to 84.60 g), in A_2 increased 100.88 g (15, 62 to 116.50 g), while in A_3 increased 132.96 g (15.50 to 148.46 g).

In Fig. 1, it was clear that the three different pH treatments seemed no influence on the total weight of the increased growth of water hyacinth. This was supported by the results of correlation with linear regression method obtained R = 0.909 at 95% confidence level. The results showed that of the three treatments, the highest increase of the pH occurred at the day 3, i.e. 6.98; 11.24; and 9.45% and the lowest occurred at day 21 1.92; 2.06; and 2.24%. Furthermore, the growth of the total weight of the highest of the three treatments occurred at the same age (day 9) by RG respectively by 1.46; 1.67; and 1.74. This indicated that optimal growth occurs at pH 5.64 to 5.91. It seemed clear that until day 9, the total weight was increasing, then it decreased even though the pH is increased to near neutral pH. This was in line with previous studies that reported that an increase in pH to near neutral does not always provide high AGR root length also (Madkar and Kurniadie, 2003: El-Gendy *et al.*, 2004: Trinidad *et al.*, 2008: Lu, 2009). However, in the pH range that can be tolerated by water hyacinth. The high pH change occurred at the day 3 may be caused by compounds that can neutralize the pH. There was more side surface of the limestone in early application. As a result, the reaction rate was higher than the following days.

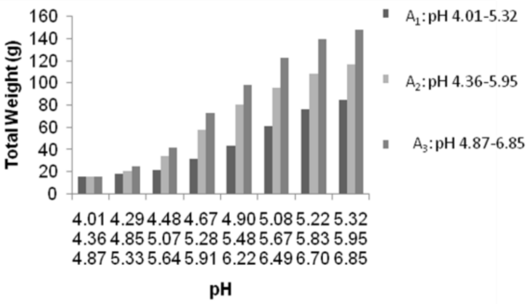

Fig. 1. Effect of pH on the Growth of Water Hyacinth with Three Treatments for 21 Days.

The high increase in the total weight at the day 9 showed the phase of plant adaptation to the growing medium has passed. Yatim (2004) explained with increasing pH, the growth of water hyacinth has increased. This phenomenon may be influenced by the other factor such as heavy metal content. Hasan *et al.* (2007) reported that high concentrations of heavy metals can inhibit the growth of water hyacinth. Other factors that may affect the growth of water hyacinth was the low content of nitrate and phosphate and AMD low pH impaired nutrient absorption (Pujawati, 2006). The decline in the growth rate of the total weight of the water hyacinth on condition perilously close to neutral pH, may be caused by the decreasing availability of nutrients. Soti and Volin (2010) reported that high nutrient lead to higher RG thus sufficient for the occurrence of asexual reproduction resulting in a higher biomass. Other studies have reported that more efficient absorption of nutrients in the young water hyacinth compared to the old, hence regular harvest old plant is very important (Ayyasamy *et al.*, 2009).

Root length

Table 1 showed that the growth of water hyacinth roots increased in all treatments as follow: A_1 along 18.95 cm (1.18 to 20.13 cm), A_2 along 24.19 cm (1, 28 to 25.47 cm), and A_3 32.65 cm (1.27 to 33.92 cm). This meant an increase in the pH of the A_1 of 1.31 or 32.67% followed by an increase in root length of 1605.93%, A_2 increase in pH of 1.59, or 36.47%, followed by an increase of 1889.84% root length, and A_3 increase in pH of 1.98 or 40.66% followed by an increase in root length of 2570.87%. This is clearly shown in Fig. 2.

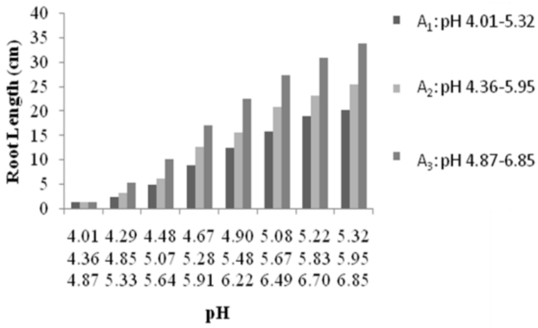

Fig. 2. Effect of pH on the Growth of Water Hyacinth Root Length of Three Treatments for 21 days.

This indicated that pH affect on the growth of water hyacinth root length. This is supported by the results of correlation with linear regression method obtained R = 0.901 and R2 = 0.811 with a confidence level of 95%. The results showed that of the three treatments, the highest increase in the pH of each occurred in the day 3, i.e. (6.98; 11.24; and 9.45%) and the lowest occurred in the day 21 (1.92; 2.06; and 2.24%). While the three treatments have absolute growth rate (AGR) for 21 days thirds occur in the day 9 respectively by 1.30; 2.18; and 2.27. This result was in line with previous studies that reported that an increase in pH to near neutral did not produce high AGR root length (Madkar and Kurniadie, 2003: El-Gendy *et al.*, 2004: Trinidad *et al.*, 2008: Lu, 2009). However, this pH range can be tolerated by water hyacinth. Thus in addition to the pH factor, there may be other factors that may affect the growth of water hyacinth roots. Rosmarkam and Yuwono (2002) reported that the phosphor element is needed to encourage the growth of root length and root hairs multiply, thus increasing the ability of plants to absorb nutrients. Plants take phosphate through assimilation by the roots into the xylem and shoots (Lambers and Colmer, 2005: Sooknah, 2000). Gupta *et al.* (2012), stated that the system with a shallow depth of more efficient in removing N and P.

In addition, Xie and Dan Yu (2003) reported that the root morphology and growth of water hyacinth are subject to availability of phosphor in the media and in significant longer roots when grown in environments containing higher phosphor. Other study by Soti and Volin (2010) stated that the high availability of nutrients will lead to higher root growth and this may be used for reproduction. Thus, the rate of decline in the availability of phosphor in the media was very likely due to the high ability of water hyacinth to absorb phosphor, hence after the day 9 root length growth rate tended to decrease even the pH was very conducive to growth. Ge Xu-guang *et al.* (2008) found that in the process of growth of water hyacinth, P accumulation increases faster than N. Several studies have reported that the water hyacinth is capable of absorbing element P in wastewater is high (Akinbile and Yusoff, 2012; Jianbo *et al.*, 2008; Aremu *et al.*, 2012; Song Wei *et al.*, 2008). Pujawati (2006), reported that AMD contain 0.042 to 0.53 ppm of phosphor. Iman (2002) stated that the optimum growth of water hyacinth occurs in P content of 20 ppm and critical levels of P by 0.1 ppm.

Thus, it was considered sufficient to support the growth of water hyacinth.

Leaves diameter

Table 1 showed that the diameter of the leaves increased in all treatment. The increase in growth since the beginning of each acquired A_1 12.82 cm wide (2.38 to 15.20 cm), A_2 18.15 cm (2.37 to 20.52 cm), and A_3 24.53 cm (2.37 to 26.90 cm). This meant an increase in the pH of the A_1 of 1.31 or 32.67% followed by an increase of 538.66% leaf diameter, A_2 increase in pH of 1.59, or 36.47%, followed by an increase of 765.82% leaf diameter, and A_3 increase in pH of 1.98 or 40.66% followed by an increase of 1035.02% leaf diameter. This was clearly shown in Fig. 3.

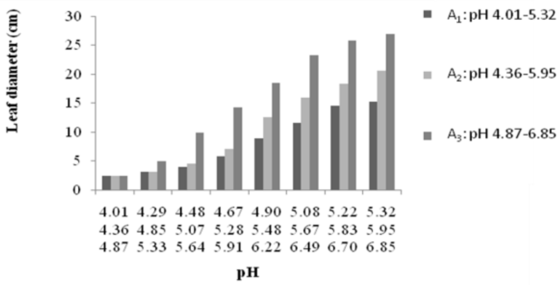

Fig. 3. Effect of pH on the Growth of Water Hyacinth Leave Diameter of Three Treatments for 21 days.

These results indicate that the effect of pH on the growth of leaves was significant. This was supported by the results of correlation with linear regression method obtained r = 0.918 at 95% confidence level. Same trend has been observed on the growth of the total weight and length root, which of the three treatments experienced the highest increase in the pH of occurred in day 3 as follows (6.98; 11.24; and 9.45%) and the lowest in the day 21 (1.92; 2.06; and 2.24%). Otherwise leaf growth at A_1 and A_2 occurs at the same age (day 12) respectively by 1.00; 1.82. In contrast, in the A_3 treatment that occurred in the the day 6 of 1.65. Thus, the change in pH closer to neutral state seemed to have a fluctuating pattern of leaf growth, after experiencing the highest growth, especially in the A_1 and A_3. This result was differ from previous studies those reported that the pH closer to neutral lead to the better for growth (Madkar and Kurniadie, 2003: El-Gendy *et al.*, 2004: Trinidad *et al.*, 2008: Lu, 2009).

Another factor may affect the availability of elements. Hill (2014) has used δ15N, where N has role in leaf and petiole growth of water hyacinth. This is in line with Rosmarkam and Yuwono (2002) study, which stated that the general element of N is required for cell division and cell enlargement. Thus the shortage of N will result in plants become stunted and yellowing. In this study, though AGR score seemed to decline after reaching the optimal, but leaf yellowing or dead did not occur until the last day of treatment. Several studies have reported that the water hyacinth is able to remove the high N from wastewater (Shah *et al.*, 2010; Dar *et al.*, 2011; Akinbile and Yusoff, 2012; Ndimele, 2012; Williams, 2009).

Pujawati (2006) found that the AMD contains of 0.69 to 2.33 ppm of nitrate. Iman (2002) states that the optimum growth of water hyacinth occurred on N content of 25 ppm. While the critical levels of N to support the growth of water hyacinth occurred at 0.03 ppm. This result was considered sufficient to support the growth of water hyacinth. Gupta *et al.* (2012) found that the system with a shallow depth had a more efficient for absorbing nitrogen.

Based on the description above, this study concluded that pH of the AMD affects the growth of water hyacinth (total weight, root length and diameter of the leaf). The reduced rate of growth of water hyacinth at a pH closer to neutral state can be due to the reduced availability of nutrients, as AMD containing low nutrients.

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